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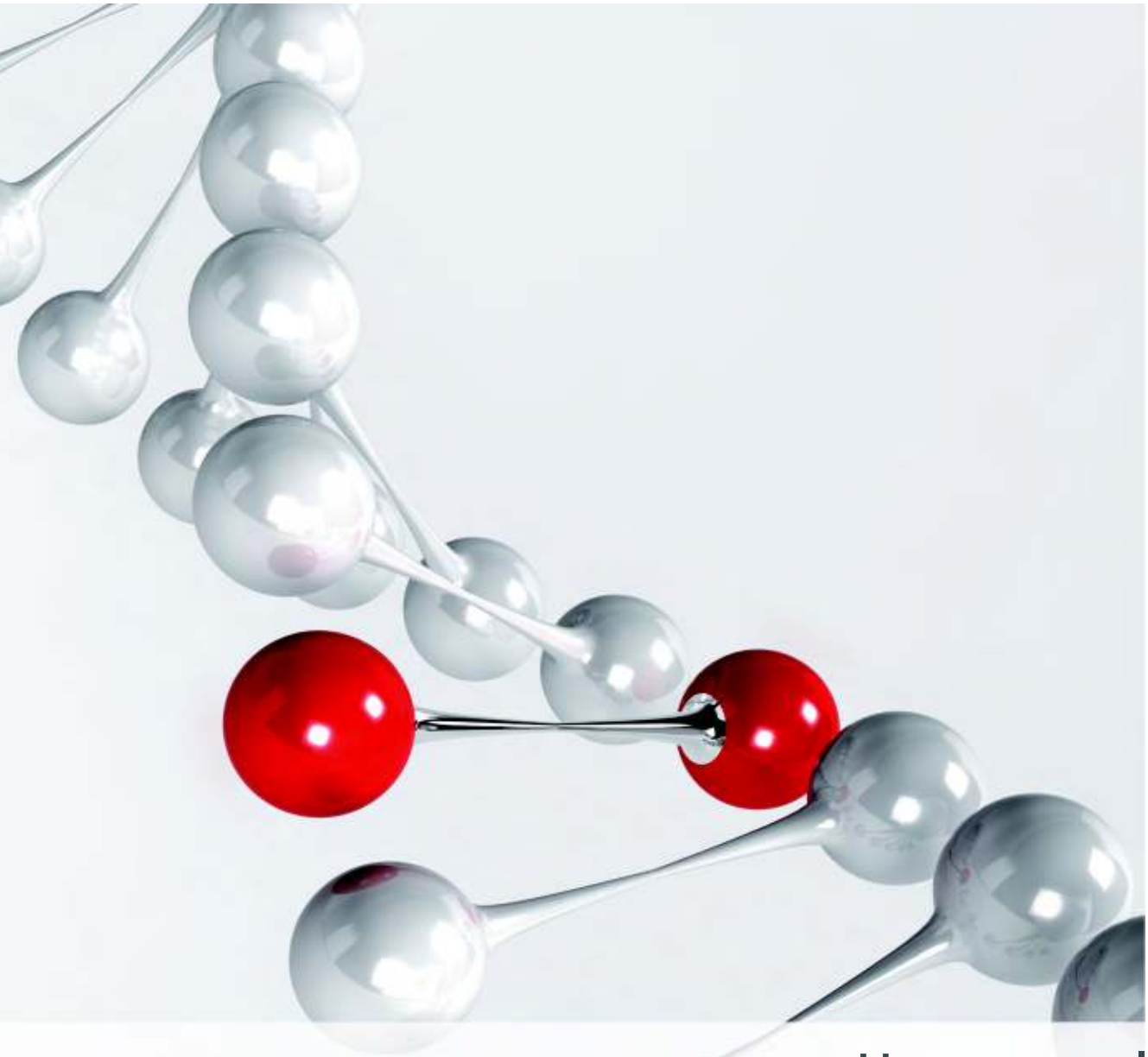

User manual
Invisorb® Spin Blood Midi Kit

For purification of genomic DNA from 200 - 2000 µl fresh or frozen whole mammalian blood (with the common anticoagulants: EDTA, Citrate)

CE
IVD

REF 1031110x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for the Invisorb® Spin Blood Midi Kit

The **Invisorb® Spin Blood Midi Kit** is the ideal tools, using the Invisorb® technology for a fast, efficient and simple manual isolation and purification of genomic DNA f from up to 2 ml/ of fresh, frozen with common anticoagulants (EDTA, Citrate) blood. The purified DNA can be used for *in vitro* diagnostic analysis.

The kit is neither validated for the isolation of genomic DNA from tissue, serum, plasma, synovial fluid and urine, nor from bacteria, stool sample, fungi, parasites or the purification of total RNA.

The application of the kit for isolation and purification of viral DNA has not been evaluated.

CE **IVD** Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Trademarks: Invisorb®, Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of the Invisorb® Spin Blood Midi Kit

Store dissolved Proteinase K at –20°C!
Store all other kit components at room temperature (RT)!

	5 DNA preps	50 DNA preps
Catalogue No.	1031110100	1031110300
Lysis Buffer A	2 x 2 ml	60 ml
Binding Buffer A	2 x 1 ml	9 ml (final volume 30 ml)
Buffer EL	30 ml (ready to use)	-
Buffer EL Concentrate	-	30 ml (final volume 1000 ml)
Proteinase K	for 250 µl working solution	for 2 ml working solution
Wash Buffer I	15 ml (ready to use)	30 ml (final volume 60 ml)
Wash Buffer II	15 ml (ready to use)	18 ml (final volume 60 ml)
Elution Buffer	2 ml	15 ml
Spin Filter	5	50
2.0 ml Receiver Tubes	10	2 x 50
1.5 ml Receiver Tubes	5	50
Manual	1	1
Initial steps	Add 250 µl ddH_2O to the tube **Proteinase K**, mix thoroughly and store at -20°C !	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix shortly and keep the bottle always firmly closed! Add 42 ml of 96-100% Ethanol to the bottle **Wash Buffer II**, mix shortly and keep the bottle always firmly closed! Mix 30 ml **Buffer EL Concentrate** with 970 ml ddH_20, mix very well and label the bottle with the Label Buffer EL Add 2 ml ddH_2O to the tube **Proteinase K**, mix thoroughly and store at -20°C !

Symbols

Manufacturer

Lot number

Catalogue number

Date of manufacture

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit components of the **Invisorb® Spin Blood Midi Kit**, except dissolved **Proteinase K** should be stored at room temperature (RT) and are stable for 12 months under these conditions. **Room temperature (RT) is defined as range from 15 - 30°C.**

Dissolved **Proteinase K** stored at –20°C is stable for 12 months, but repeated freezing and thawing should be avoided. Aliquotation and storage at – 20°C is recommended.

Wash Buffer charged with ethanol should be appropriately sealed and stored at room temperature.

If there are any precipitates within the provided solutions dissolve these precipitates by carefully warming up to room temperature (up to 30°C).

Quality control and product warranty.

STRATEC Molecular warrants the correct function of the **Invisorb® Spin Blood Midi Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **Invisorb® Spin Blood Midi Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **Invisorb® Spin Blood Midi Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **Invisorb® Spin Blood Midi Kit** is the ideal tool for a fast and convenient manual isolation and purification of genomic DNA from max. 2 ml fresh, frozen or old human blood.
For reproducible and high yields appropriate sample storage is essential. The purified DNA can be used for *in vitro* diagnostic analysis only.

Fresh or frozen whole blood treated with EDTA or citrate, but not with heparin, from common blood collection systems can be used.

The protocols for the isolation and all buffers are optimized for a high yield as well as a high purity. All hands on steps are reduced to a minimum.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from tissue, serum, plasma, synovial fluid and urine, nor from bacteria, stool sample, fungi, parasites or the purification of total RNA

The application of the kits for isolation and purification of viral DNA has not been evaluated. The kits applicability for cultured or isolated cells, tissue, stool sample, swabs, dried blood stains, or cell free body fluids, like cerebrospinal fluid, synovial fluid, and urine, also have not been validated.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **Invisorb® Spin Blood Midi Kit** procedure for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered potentially infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb® Spin Blood Midi Kit** to which they apply, are listed below as follows:

Lysis Buffer A

danger
H319 P305-351-338

Proteinase K

danger
H315-319-334-335 P280-305-351-338

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

H319:	Causes serious eye irritation.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P273:	Avoid release to the environment.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: **1 – 352 – 323 – 3500**
in USA : **1 – 800 – 535 – 5053**

Product characteristic

Starting material/ Invisorb® Spin Blood Midi Kit	Yield	Time for preparation	Ratio
200 µl up to 2 ml fresh whole blood samples, frozen, old or dry blood	up to 50 µg (depends on amount and specify of starting material)	about 30 minutes	A_{260} : A_{280} 1.6 – 2.0

The **Invisorb® Spin Blood Midi Kit** provides a very efficient procedure for isolation of high quality DNA directly from fresh, frozen, or old blood samples treated with citrate or EDTA
The kit is designed for simultaneous processing of multiple samples.
After lysis of erythrocytes, lymphocytes can be isolated free of any interfering hemoglobin.
The purification procedure of the kit is rapid and avoids the use of chaotropic components.
The DNA binds to filter membrane, followed by washing steps and the final elution.
The purification procedure is rapid and requires neither phenol / chloroform extraction nor alcohol precipitation, and requires minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination. Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at –20°C for subsequent use.

Downstream Application:

- PCR *)
- real-time PCR
- RFLP-Analysis
- Restriction Enzyme Digestion
- Southern Blot Analysis
- Sequencing
- Cloning
- HLA typing
- STR-Analysis
- SNP-Analysis

To purify genomic DNA in 96 format STRATEC Molecular offers the **Invisorb® Blood Mini HTS 96 Kit** for use in a centrifuge and on common laboratory automated workstations. Furthermore STRATEC Molecular offers the **InviMag® Blood Midi Kits** for DNA isolation using magnetic beads.
For minimal amount of starting material the **Invisorb® Spin Blood Mini Kit** (max. 200 µl) is available. To purify genomic DNA from large volumes of blood STRATEC Molecular offers the **Invisorb® Blood Universal Kit** (1 – 10 ml).
For further information please contact: Tel.: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries Tel.: +49 (0) 30 9489 2907 or your local distributor.

*) The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Principle and procedure

The **Invisorb® Spin Blood Midi Kit** procedure comprise following steps:

1. lysis of erythrocytes
2. lysis of nucleus containing cells
3. binding the genomic DNA to the membrane of Spin Filter
4. washing the membrane and elimination of ethanol
5. elution of genomic DNA

This manual contains 2 protocols, according to the different requirements of the starting materials.

Sampling and storage of starting material

Blood

Blood samples (stabilized with EDTA or Citrate) can be stored at room temperature for 2 - 3 hours, for short time storage (up to 24 h) samples may be stored at – 4 °C. For long term storage, we recommend freezing samples at – 20°C or – 80°C. Multiple thawing and freezing before isolating the DNA should be avoided. If cryoprecipitate (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the Spin Filter membrane. Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Procedure

Lysis of Erythrocytes and pelleting down the nucleus containing cells

Lysis of the the nucleus containing cells

Samples are lysed at elevated temperatures. Lysis is performed in the presence of **Lysis Buffer A** and **Proteinase K.**

Binding genomic DNA

By adding **Binding Buffer A** to the lysate, optimal binding conditions will be adjusted. Each lysate is then applied to a Spin Filter and genomic DNA is adsorbed to the membrane.

Removing residual contaminations

Contaminants are efficiently washed away using **Wash Buffers**, while the genomic DNA remains bound to the membrane.

Elution

Genomic DNA is eluted from the Spin Filter using **Elution Buffer**. The eluted DNA is ready for use in different downstream applications. Eluted DNA stored at 4 – 8°C is stable for 2 months, for more than 5 years if stored at -20°C.

Yield and quality of genomic DNA

The amount of purified DNA using the **Invisorb® Spin Blood Midi Kit** procedure, depends on the sample type and the number of cells in the sample (depending from the patients age and health situation, sample source, transport conditions, storage, and age of the sample).

Typically, a 2 ml sample of whole blood cells from a healthy individual will yield up to 60 µg of DNA. Samples with elevated white blood cell give a higher yield.

For most whole blood samples, a single elution with 200 µl **Elution Buffer** is sufficient. For samples with elevated white blood cell approximately 80% of the DNA will elute in the first 200 µl, and up to 20% more in the next 200 µl.

Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturers' specifications.

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers of the Invisorb® Spin Blood Midi Kit

1. Adjust the thermo mixer to 70°C.
2. Warm up the needed amount of **Elution Buffer** to 70°C
   (200 µl **Elution Buffer** are needed per sample).
3. Label the needed amount of Spin Filters.
4. Label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube).
5. Add the needed µl ddH_2O to reaction tube with **Proteinase K** (see below). Vortex for 5 s.
6. Add the needed amount of ethanol to the **Wash Buffers**.

5 DNA extractions:
add 250 µl ddH_2O to **Proteinase K**, mix thoroughly (vortex 5s) and store at –20°C **Wash Buffers**, **Binding Buffer A** and **Buffer EL** are ready to use
50 DNA extractions:
add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. add 2 ml ddH_2O to **Proteinase K**, mix thoroughly (vortex 5s) and store at-20°C mix 30 ml **Buffer EL Concentrate** with 970 ml ddH_20, mix very well and label the bottle with the Label Buffer EL add 30 ml of 96 - 100 % ethanol to **Wash Buffer I** add 42 ml of 96 - 100 % ethanol to **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed

Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com under each STRATEC Molecular kit and kit component.

- Microcentrifuge
- Thermomixer (for 70°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipette and pipette tips
- Vortexer
- Reaction tubes (1.5 ml or 2.0 ml)
- ddH_2O
- 96 - 100 % ethanol
- 1 x PBS (optional)
- Isopropanol*

*The **Invisorb® Spin Blood Midi Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Important indications

1. Process only as much blood samples as the micro centrifuge allows to process.

2. Blood sample and buffers should be thoroughly mixed and should have room temperature

3. The elution can be done by using lower amount of **Elution Buffer**. This may result in a higher concentration of DNA. But pay attention that minimum volume for elution is 50 µl, but this will reduce the yield. Elution volume between 2 x 50 µl up to 200 µl will realize comparable results.

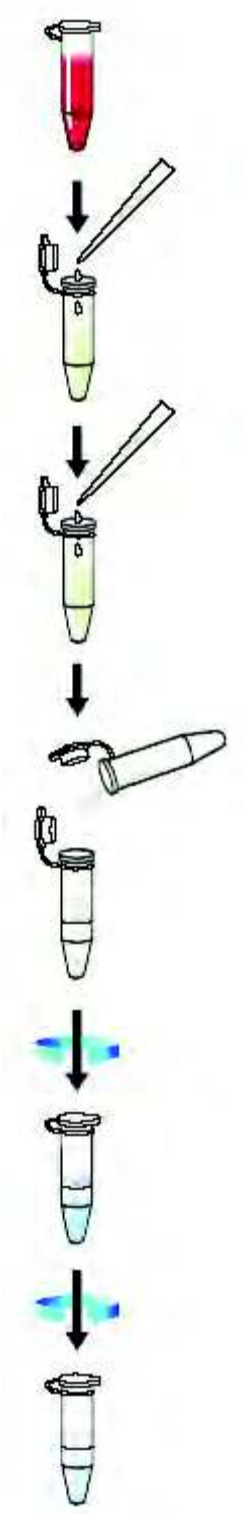
4. The eluted DNA volume will be lower than the added **Elution Buffer** volume. **Elution Buffer** should be preheated to 70 °C.

5. The **Elution Buffer** doesn't contain EDTA.

6. The yield can be increased, if the incubation time with preheated **Elution Buffer** will be prolonged.

7. Old blood samples often contains coagulates, if coagulates or cryoprecipitate (formed during thawing of frozen samples) are visible avoid aspirating them, they could clog the Spin Filter membrane.

Scheme of the Invisorb® Spin Blood Midi Kit



genomic DNA

Please read protocols prior the start of the preparation carefully

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Transfer. 200 µl up to max 1000 µl of blood into a 2 ml Receiver Tube
add 1 ml of cold Buffer EL (4°C); mix shortly and incubate for 5 min
Centrifuge at 3.500 x g (5.000 rpm) for 1 minute, discard the supernatant

Add 400 µl **Lysis Buffer A** and **40 µl Proteinase K,** vortex shortly,
Incubate for 20 min at 70°C while continuously shaking

Add 200 µl **Binding Buffer A** *(follow preparing instructions)* and vortex shortly

Transfer the solution onto Spin Filter, incubate for 1 min
Centrifuge for 2 min at 11.000 x g (11.000 rpm)
Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube

Add 500 µl **Wash Buffer I**
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube

Add 550 µl **Wash Buffer II**
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Discard the filtrate, place the filter again into the Receiver Tube, repeat the step.
Discard the filtrate, put the filter back into the 2.0 ml Receiver Tube
then centrifuge for 4 min at maximum speed for ethanol removal

Place the Spin Filter into a 1.5 ml Receiver Tube
Add 100 µl **Elution Buffer** (preheated to 70°C)
Incubate for 3 min at room temperature
Centrifuge for 1 min at 11.000 x g (11.000 rpm)
Add again 100 µl of the Elution Buffer
Centrifuge for 1 min at 11.000 x g (11.000 rpm)

Discard Spin Filter
Close the 1.5 ml Receiver Tube and store the DNA sample at 4°C or –20°C

Protocol 1: DNA extraction from 200 µl up to 1 ml blood

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 8*

Important *Transfer the needed amount of* ***Elution Buffer*** *into 2.0 ml Receiver Tube (not included in the kit) and place the tube at 70°C.*

1. Lysis of Erythrocytes and pelleting down the nucleus containing cells
Transfer of 200 µl up to max. 1 ml of the blood sample into a 2.0 ml reaction tube and add 1 ml of cold Buffer EL (4°C); mix shortly and incubate for 5 min.
Centrifuge at 3.500 x g (5.000 rpm) for 1 minute (RT). Carefully discard the supernatant. If the lymphocyte pellet have a red color please add 1 ml of Buffer EL, vortex shortly and centrifuge at 3.500 x g (5.000 rpm) for 1 minute again. If there is still a red color of the pellet, please wash again with Buffer El. Carefully discard the supernatant

Important: ***Don't remove the cell pellet!***
Please note, if you work with a fresh blood sample (up to 4 hours after taking of the sample) extend the lysis of erythrocytes by 30 minutes (total time at least 45 min).

2. Lysis at 70°C for 20 min in a Thermo mixer
Add 400 µl Lysis Buffer A and 40 µl Proteinase K, vortex shortly and incubate in a shaking Thermo mixer at 70°C for 20 min. Place a Spin Filter into a 2.0 ml Receiver Tube.

3. Realizing of optimal binding conditions
Add 200 µl Binding Buffer A and vortex shortly.

4. Loading the DNA binding Spin Filter
Transfer the solution onto the Spin Filter and incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) (table centrifuge) for 2 min. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

5. First Washing step
Add 500 µl Wash Buffer I and centrifuge at 11.000 x g (11.000 rpm) for 1min. Discard the filtrate, place the filter back into the 2.0 ml Receiver Tube.

6. Second Washing step
Add 550 µl Wash Buffer II and centrifuge at 11.000 x g (11.000 rpm) for 1min. Discard the filtrate, place the filter again into the Receiver Tube. Repeat the washing step once again. Discard the filtrate, put the filter back into the 2.0 ml Receiver Tube and finally centrifuge for 4 min at maximum speed to remove the residual ethanol completely.

7. Elution of the DNA
Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 µl of preheated Elution Buffer. Incubation for 3 min. Centrifuge for 1 min at 11.000 x g (11.000 rpm). Add again 100 µl of the Elution Buffer and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.

Note: *The DNA can also be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention, that the minimum volume for the elution is 50 µl. If a quite large amount of DNA is expected, the volume of elution can be increased (100-200 µl****).***
To maximize the final yield we recommend ever the use of two elution steps with an equal volume of Elution Buffer.

Protocol 2: DNA extraction from 1 ml – 2 ml blood

Please read the instructions carefully and conduct the prepared procedure.

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 8*

Important *Transfer the needed amount of* ***Elution Buffer*** *into 2.0 ml Receiver Tube (not included in the kit) and place the tube at 70°C.*

1. Lysis of Erythrocytes and pelleting down of the nucleus containing cells
Transfer of the blood sample into a 15 ml centrifuge tube and add 5 ml of cold Buffer EL (4°C); mix shortly and incubate for 5 min.
Centrifuge at 2.250 x g (4.000 rpm) for 5 minutes. Carefully discard the supernatant. Add again 5 ml of Buffer EL to the cell pellet and centrifuge at 3.500 x g (5.000 rpm) for 5 minutes. Carefully discard the supernatant. If there is still a red color of the pellet, please wash again with Buffer El. Carefully discard the supernatant

Important: ***Don't remove the cell pellet!***
Please note, if you work with a fresh blood sample (up to 4 hours after taking of the sample) extend the lysis of erythrocytes by 30 minutes (total time at least 45 min).

2. Lysis at 70°C for 20 min in a thermo mixer
Add 600 µl Lysis Buffer A to the cell pellet. Pipett sometimes up and down and transfer the lysate completely into a 2.0 ml Receiver Tube. Add 40 µl Proteinase K, vortex shortly and incubate in a thermo mixer at 70°C for 20 min. Place a Spin Filter into a 2.0 ml Receiver Tube.

3. Realizing of optimal binding conditions
Add 300 µl Binding Buffer A and vortex shortly.

4. Loading the DNA binding Spin Filter
Transfer 600 µl of the lysate onto the Spin Filter and incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.
Reload the Spin Filter with the residual lysate and repeat the centrifugation step. Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

5. First Washing step
Add 500 µl Wash Buffer I and centrifuge at 11.000 x g (11.000 rpm) for 1min. Discard the filtrate, place the filter back into the 2.0 ml Receiver Tube.

6. Second Washing step
Add 550 µl Wash Buffer II and centrifuge at 11.000 x g (11.000 rpm) for 1min. Discard the filtrate, place the filter again into the Receiver Tube. Repeat the washing step once again. Discard the filtrate, put the filter back into the 2.0 ml Receiver Tube and finally centrifuge for 4 min at maximum speed to remove residual ethanol completely.

7. Elution of the DNA
Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100 µl of preheated Elution Buffer. Incubation for 3 min. Centrifuge for 1 min at 11.000 x g (11.000 rpm). Add again 100 µl of the Elution Buffer and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.

Note: ◆ *The DNA can also be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention, that the minimum volume for the elution is 50 µl. If a quite large amount of DNA is expected, the volume of elution can be increased (100-200 µl)* ***. To maximize the final yield we recommend ever the use of two elution steps with an equal volume of Elution Buffer.***

Troubleshooting

Problem	Cause	Comments and suggestions
clogged Spin Filter	insufficient lysis and/or too much starting material	increase lysis time increase centrifugation speed or time reduce amount of starting material
low amount of extracted DNA	insufficient lysis incomplete elution insufficient mixing with **Binding Buffer A**	increase lysis time reduce amount of starting material overloading of Spin Filter reduces yield prolong the incubation time with **Elution Buffer** to 5-10 min or repeat elution step once again take higher volume of **Elution Buffer** Mix sample with Binding Buffer A by pipetting or by vortexing prior to transfer the sample onto the Spin Filter
Spin Filter surface turns yellow	incomplete lysis insufficient washing too old starting material	see above wash again with **Wash Buffer II** use protocol 2
low concentration of extracted DNA	too much Elution Buffer	elute the DNA with lower volume of **Elution Buffer**
degraded or sheared DNA	incorrect storage of starting material old material	ensure that the starting material is fresh or stored under appropriate conditions (for long time storage at – 20°C) avoid thawing and freezing of the material old material often contains degraded DNA
genomic DNA does not perform well in downstream-applications	ethanol carryover during elution Salt carryover during elution	Increase centrifugation time for of ethanol removal ensure that **Wash Buffer** is at room temperature check up **Wash Buffer** for salt precipitates. If there are any precipitates, dissolve these precipitates by careful warming

Appendix

General notes on handling DNA

Starting material

This kit is designed for extraction of DNA from blood, but even human blood is different between individuals depending on age, health, and conditions of life. If you are using blood from animals keep in mind that lyses conditions of blood differ depending on the species. Also remember that non-mammalian blood contains erythrocytes with nuclei. So for special applications adaptation of starting volumes and lyses time may be recommended.

Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting and long-template PCR.

Storage of DNA

A working stock of DNA can be stored at 2 – 4˚C for several weeks. For long term storage DNA should be stored at -20˚C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA

Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA Yield

The amount of purified DNA from the whole blood depends on the leucocytes content, sample source, transport, storage, and age. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Ordering information

Product	Package size	Catalogue No.
Invisorb® Spin Blood Midi Kit	5 preparations	1031110100
Invisorb® Spin Blood Midi Kit	50 preparations	1031110300

Single components for the Invisorb® Spin Blood Midi Kit

Lysis Buffer A	60 ml	1031111100
Binding Buffer A	30 ml	1031112100
Buffer EL (concentrate)	30 ml	1031115600
Wash Buffer I (add 30 ml)	30 ml	1031113300
Wash Buffer II (add 42 ml)	18 ml	1031113400
Elution Buffer	15 ml	1031114000

Related products

Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
Invisorb® Blood Universal Kit	500 ml	1031150200
Invisorb® Blood Universal Kit	1000 ml	1031150300
Invisorb® Blood Mini 96 HTS/ C	4 x 96 preparations	7031300300
Invisorb® Blood Mini 96 HTS/ C	24 x 96 preparations	7031300400
Invisorb® Blood Mini 96 HTS/ R	4 x 96 preparations	7131300300
Invisorb® Blood Mini 96 HTS/ R	24 x 96 preparations	7131300400
InviMag® Blood DNA Mini Kit/ KFml	15 preparations	1031160100
InviMag® Blood DNA Mini Kit/ KFml	75 preparations	1031160200
InviMag® Blood DNA Mini Kit/ KF96	1 x 96 preparations	7431300100
InviMag® Blood DNA Mini Kit/ KF96	5 x 96 preparations	7431300200

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1A3a02/04/2013